PATENT
ATTORNEY DOCKET NO. 50195/023003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	James M. Robl et al.	Confirmation No.:	4828
Serial No.:	10/705,519	Art Unit:	1632
Filed:	November 10, 2003	Examiner:	Deborah Crouch
Customer No.:	21559		

Title: TRANSGENIC UNGULATES HAVING REDUCED PRION PROTEIN ACTIVITY AND USES THEREOF

DECLARATION OF DR. YOSHIMI KUROIWA TRAVERSING GROUNDS OF REJECTION OVER GOOD

Under 37 C.F.R. § 1.132 and regarding the rejection for anticipation by Good et al. (U.S. Patent Publication No. 2002/0069423; hereafter "Good") in the Office Action mailed August 9, 2007, I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application. My curriculum vita is of record.

2. I have considered the statement by the Office, "at the time of filing, transfected fetal fibroblasts had been isolated in the art by culturing transfected fibroblasts in the presence of selection medium followed by isolation with cloning rings ([Bondioli, et al. Mole. Reproduct. Develop., 2001, 60] page 190. col. 1, parag. 2, lines 10-24)," and disagree with the Office's conclusions. The Bondioli reference attempted to introduce a puromycin resistance gene into fibroblasts from a transgenic boar (page 190, first column). The authors then used cells isolated using cloning rings from puromycin-containing medium to produce cloned piglets (pages 190-191). PCR analysis of the cloned piglets indicated, however, that they did not contain the puromycin resistance gene (page 191, second column). Thus,

the authors of the reference failed to produce a correctly targeted pig using cells isolated by cloning rings.

In general, it is known that selecting transgenic somatic cells by antibiotics does not always produce the expected transgenic cloned animals. This phenomenon is called the "bystander effect," where transgenic cells expressing an antibiotic-resistance gene provide protection to nearby non-transgenic cells either by secretion of the gene product into the medium or by direct cell-to-cell contact. Consequently, many transfected colonies are mixed and contain both transgenic and non-transgenic cells.

3. I have also considered the statement by the Office, "all there needs to be [to produce a live cow] is one bovine fetal fibroblast containing a nonfunctional PrP gene, which can then be grown to produce a cell line" I also disagree with this statement. As noted in my declaration filed on June 2007, ¶4, not all correctly targeted cell lines produce live offspring, even though they may produce pregnancies. In my experience, cloning efficiency for producing live animals is approximately 10%. Accordingly, one would typically require ten cell lines to produce a live animal. Thus, in my opinion, neither I nor other scientists in this area would believe that a single, correctly targeted cell would be sufficient to produce live offspring.

4. With respect to determining whether heterozygous cells have been correctly targeted to produce homozygous cells, I have also considered the statement by the Office, "It is possible, that the second targeting vector will insert at the second prion locus. Thus, cells that are resistant to both antibiotics would have a reasonable expectation of having both loci targeted." In general, somatic cells, like transgenic fibroblasts, are fragile. I am not aware of anyone using simultaneous selection against two antibiotics for selection of somatic cells in animal cloning. In my opinion, such a selection process would be unsuccessful.

5. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

08 Feb 2008
Date

Dr. Yoshimi Kuroiwa